

1. A method for the determination of a nucleic acid molecule A, comprising (a) forming a triple stranded binding complex having a triple stranded region between said nucleic acid A, a nucleic acid A binding molecule B having a base sequence and a binding region and at least one nucleic acid A binding molecule C having a binding region and a base sequence different from the base sequence of molecule B, said triple stranded complex between molecules A, B and the at least one molecule C being more thermostable than a triple stranded complex formed by two nucleic acid A binding molecules B with one molecule of nucleic acid A or two identical nucleic acid A binding molecules C with one molecule of nucleic acid A and (b) determining the amount of said nucleic acid A by measuring the amount of said triple stranded binding complex.
2. A method according to claim 1 wherein said triple stranded complex contains a triple helical binding region to which each of molecules A, B and C contribute with at least 1 but less than 11 bases.
3. A method according to claim 1 wherein said complex contains one nucleic acid A binding molecule C binding in the triple stranded region.
4. A method according to claim 3 wherein said nucleic acid A binding molecule B has a base sequence participating in binding in the triple stranded region of more than 3 but less than 11 bases.
5. A method according to claim 1 wherein the binding region of said nucleic acid A binding molecule B is smaller in length than the base sequence bound by molecule C.
6. A method according to claim 1 wherein said nucleic acid A binding molecule C has a length of at least 6 bases.
7. A method according to claim 1 wherein the triple stranded binding complex formed contains two different molecules C binding in the triple stranded region.

8. A method according to claim 7 wherein the nucleic acid A binding molecules C bind to nucleic acid A adjacently.
9. A method according to claim 1 wherein at least one of said nucleic acid A binding molecules B and C has been chemically modified to destabilize triple helix formation of at least one of (a) two nucleic acid A binding molecules B and (b) two identical nucleic acid A binding molecules C, with one nucleic acid molecule A.
10. A method according to claim 1 wherein the binding region of nucleic acid A binding molecule B has an asymmetric base sequence.
11. A method according to claim 1 wherein the binding region of nucleic acid A binding molecule B has a symmetric base sequence.
12. A method according to claim 1 wherein nucleic acid A binding molecule B is bound to nucleic acid A via Hoogsteen base pairing and nucleic acid A binding molecule C is bound via Watson Crick base pairing.
13. A method according to claim 1 wherein at least one of nucleic acid A binding molecules, B and C is labelled and the presence of a label in the triple stranded binding complex is used for the determination of the nucleic acid.
14. A method according to claim 1 wherein at least one of nucleic acid binding molecules B and C is a nucleic acid analogue.
15. A method according to claim 14 wherein the nucleic acid analogue is a PNA.
16. A method according to claim 1 wherein the bases in the binding region of nucleic acid A binding molecule B to nucleic acid A consist of pyrimidines.
17. A method according to claim 1 wherein a first nucleic acid not to be determined is

differentiated from a second nucleic acid to be determined by a difference in the base sequence located within the region of binding of nucleic acid A binding molecule B to the first and second nucleic acids.

18. A method according to claim 1 wherein a first nucleic acid not to be determined is differentiated from a second nucleic acid to be determined by a difference in the base sequence located within the region of binding of nucleic acid A binding molecule C to the first and second nucleic acids.

19. A method according to claim 1 wherein nucleic acid A binding molecule C spans fully the region of binding of nucleic acid A binding molecule B to the nucleic acid.

20. A method according to claim 1 wherein there are used at least two different nucleic acid A binding molecules C, each of them having a different binding region which does not overlap and each binding to a part of the nucleic acid A within the region of binding of nucleic acid A binding molecule B.

21. A method according to claim 1 wherein a reaction mixture is used for forming the triple stranded binding complex, the reaction mixture containing a competitive probe molecule D which can compete with at least one nucleic acid A binding molecule C in binding to A, but which is incapable of participating in the triple stranded binding complex.

22. A triple stranded binding complex comprising a complex between a nucleic acid A, a nucleic acid A binding molecule B having a binding region and a base sequence and at least one nucleic acid A binding molecule C having a binding region and a base sequence different than nucleic acid A binding molecule B, the triple stranded binding complex being more thermostable than a triple stranded complex formed from two nucleic acid A binding molecules of B or two identical nucleic acid A binding molecules of C, with one molecule A.

23. A complex according to claim 22 wherein nucleic acid A binding molecule B is smaller in length than the overall binding region of the at least one nucleic acid A binding

molecule C and nucleic acid A binding molecule B has a base sequence of more than 3, but less than 11 bases, and the sequence of B is asymmetric.

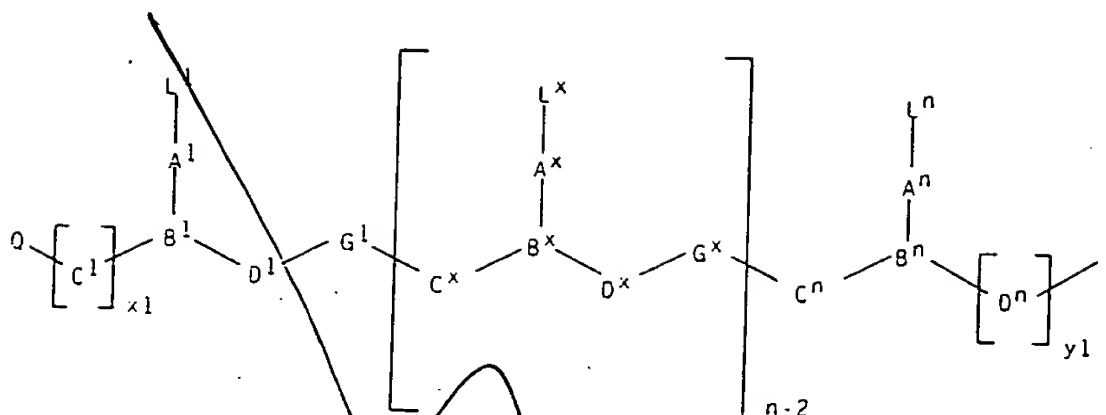
24. A complex according to claim 22 wherein nucleic acid A binding molecule B is smaller in length than the overall binding region of the at least one nucleic acid A binding molecule C and nucleic acid A binding molecule B has a base sequence of more than 3, but less than 11 bases, and the sequence of B is symmetric.

25. A method of forming a triple stranded binding complex comprising reacting a nucleic acid molecule A with a nucleic acid A binding molecule B having a base sequence and a binding region at least one nucleic acid A binding molecule C having a binding region and a base sequence different from the base sequence of molecule B, the triple stranded complex between molecules A, B and the at least one molecule C being more thermostable than a triple stranded complex formed by two nucleic acid A binding molecules with one molecule of nucleic acid A or two identical nucleic acid A binding molecules C with one molecule of nucleic acid A.

26. A method according to claim 2 wherein each of molecules A, B and C contribute more than 1 but less than 8 bases.

27. A method according to claim 4 wherein molecule B has a base sequence of more than 4 but less than 8 bases.

28. A method according to claim 1 wherein at least one of said nucleic acid A binding molecule B and nucleic acid A binding molecule C comprises a molecule of general Formula I



Formula I

wherein

n is an integer of at least 3,

x is an integer from 2 to $n-1$,

each of L^1-L^n is a ligand independently selected from the group consisting of hydrogen, hydroxy, (C_1-C_4) alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L^1-L^n is a non-nucleobase electron acceptor or a donor moiety and at least 2 of L^1-L^n a nucleobase binding group, or a naturally or non-naturally occurring nucleobase,

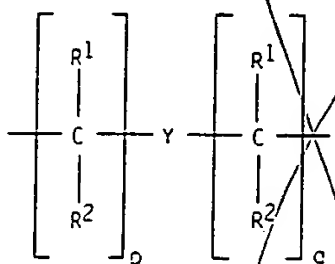
each of C^1-C^n is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_1-C_6) alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6) alkoxy, (C_1-C_6) alkylthio, NR^3R^4 and SR^5 , where R^3 and R^4 are as defined below, and R^5 is hydrogen, (C_1-C_6) alkyl, hydroxy, (C_1-C_6) alkoxy, or (C_1-C_6) alkylthio-substituted (C_1-C_6) alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system; or C^1-C^n is CO, CS, or CNR^3 ;

each of D^1-D^n is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above,

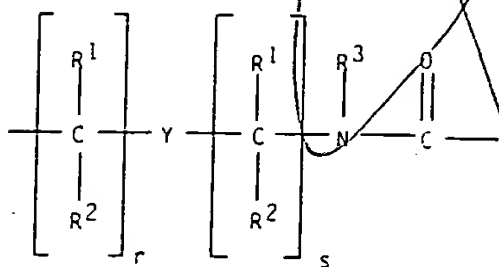
each of y and z is zero or an integer from 1 to 10, the sum $y + z$ being at least 2,
 each of G^1-G^{n-1} is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO_2-$, in either orientation, where R^3
 is as defined below;

each of A^1-A^n and B^1 and B^n are selected such that:

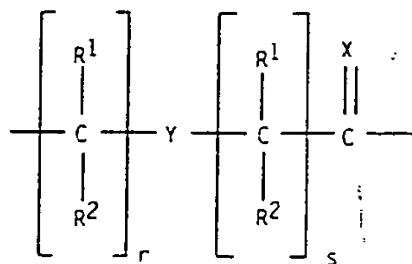
- (a) A^1-A^n is a group of formula (II/A), (II/B), (II/C) or (II/D), and B^1-B^n is N or R^3N+ or
 (b) A^1-A^n is a group of formula (II/D) and B^1-B^n is CH_3 ;



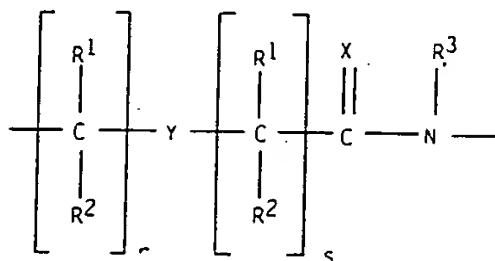
Formula II/A



Formula II/C



Formula II/B



Formula II/D

wherein:

X is O, S, Se, NR^3 , CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR^4 ,

each of p and q is zero or an integer from 1 to 5,

each of r and s is zero or an integer from 1 to 5,

each of R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4) alkyl which may be hydroxy- or (C_1-C_4) alkoxy- or (C_1-C_4) alkylthio-substituted, hydroxy, (C_1-C_4) alkoxy, (C_1-C_4) alkylthio, amino and halogen,

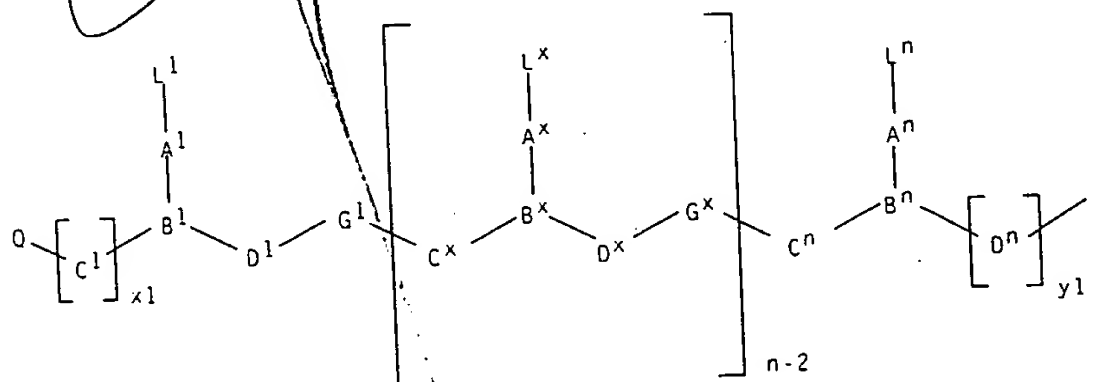
each R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4) alkyl, hydroxy-or alkoxy-or alkylthio-substituted (C_1-C_4) alkyl, hydroxy, (C_1-C_6) -alkoxy, (C_1-C_6) -alkylthio and amino,

Q and I is independently selected from $-\text{CO}_2\text{H}$, $-\text{CONR}^{\text{I}}\text{R}^{\text{II}}$, $-\text{SO}_3\text{H}$ or $-\text{SO}_2\text{NR}^{\text{I}}\text{R}^{\text{II}}$ or an active derivative of $-\text{CO}_2\text{H}$ or $-\text{SO}_3\text{H}$ and $-\text{NR}^{\text{I}}\text{R}^{\text{III}}$

wherein R^{I} , R^{II} and R^{III} are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers and as well as nucleic acid binding moieties and

each of x_1 and y_1 is an integer of from 0 to 10.

29. A method according to claim 1 wherein at least one of said nucleic acid A binding molecule B and nucleic acid A binding molecule C comprises a molecule of general Formula I



I

Formula I

wherein

n is an integer of at least 3,

x is an integer from 2 to $n-1$,

each of L^1 - L^n is a ligand independently selected from the group consisting of hydrogen, hydroxy, $(\text{C}_1\text{-C}_4)$ alkanoyl, naturally occurring nucleobases, non-naturally occurring

nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L^1-L^n is a non-nucleobase electron acceptor or a donor moiety and at least 2 of L^1-L^n a nucleobase binding group, or a naturally or non-naturally occurring nucleobase,

each of C^1-C^n is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_1-C_6) alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6) alkoxy, (C_1-C_6) alkylthio, NR^3R^4 and SR^5 , where R^3 and R^4 are as defined below, and R^5 is hydrogen, (C_1-C_6) alkyl, hydroxy, (C_1-C_6) alkoxy, or (C_1-C_6) alkylthio-substituted (C_1-C_6) alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system; or C^1-C^n is CO , CS , or CNR^3 ,

each of D^1-D^n is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above,

each of y and z is zero or an integer from 1 to 10, the sum $y + z$ being at least 2,

each of G^1-G^{n-1} is $-NR^3CO-$, $-NR^3CS-$, $-NR^3SO-$ or $-NR^3SO^2-$, in either orientation, where R^3 is as defined below;

each of A^1-A^n and B^1-B^n are selected from (Ia), (Ib) or (Ic) such that:

(Ia): B^1-B^n is N and A^1-A^n is $-CO-(CH_2)_6-$

(Ib): B^1-B^n is N and A^1-A^n is $-CO-NR^3-(CH_2)_2-$

(Ic): B^1-B^n is CH and A^1-A^n is $-NR^3-CO-(CH_2)_2-$

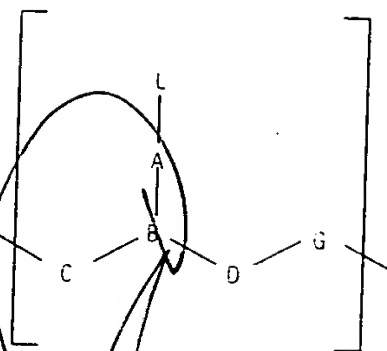
Q and I is independently selected from $-CO_2H$, $-CONR^1R^{11}$, $-SO_3H$ or $-SO_2NR^1R^{11}$ or an active derivative of $-CO_2H$ or $-SO_3H$ and $-NR^1R^{11}$

wherein R^1 , R^{11} and R^{111} are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers and as well as nucleic acid binding moieties and

each of x_1 and y_1 is an integer of from 0 to 10.

30. A method according to claim 1 wherein at least one of said nucleic acid A binding molecule B and nucleic acid A molecule C comprises a molecule of general Formula

III



Formula III

wherein

n is an integer of at least 3,

x is an integer from 2 to $n-1$,

each of L is a ligand independently selected from the group consisting of hydrogen, hydroxy, (C_1-C_4) alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L is a non-nucleobase electron acceptor or a donor moiety and at least 2 of L a nucleobase binding group, or a naturally or non-naturally occurring nucleobase,

each of C is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_1-C_6) alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6) alkoxy, (C_1-C_6) alkylthio, NR^3R^4 and SR^5 , where R^3 and R^4 are as defined below, and R^5 is hydrogen, (C_1-C_6) alkyl, hydroxy, (C_1-C_6) alkoxy, or (C_1-C_6) alkylthio-substituted (C_1-C_6) alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system; or C is CO , CS , or CNR^3 ;

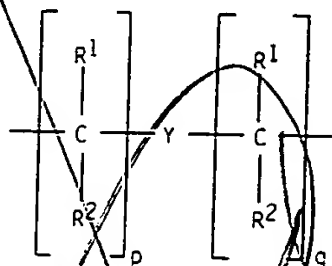
each of D is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above,

each of y and z is zero or an integer from 1 to 10, the sum y + z being at least 2,
each of G is $-\text{NR}^3\text{CO}-$, $-\text{NR}^3\text{CS}-$, $-\text{NR}^3\text{SO}-$ or $-\text{NR}^3\text{SO}_2-$, in either orientation, where R^3 is as
defined below;

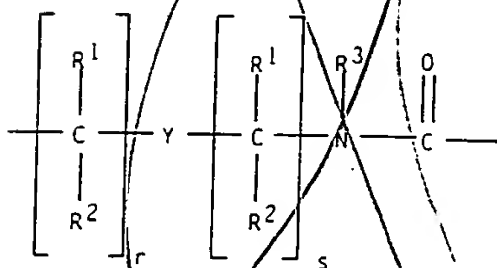
each of A and B are selected such that:

(a) A is a group of formula (II/A), (II/B), (II/C) or (II/D), and B is N or R^3N^+ or

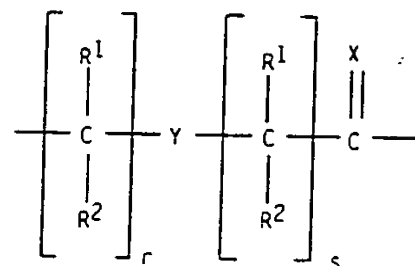
(b) A is a group of formula (II/D) and B is CH ;



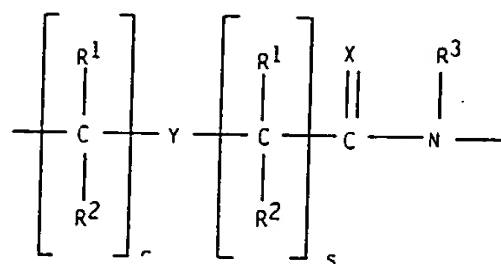
Formula II/A



Formula II/C



Formula II/B



Formula II/D

wherein:

X is O, S, Se, NR^3 , CH_2 or $\text{C}(\text{CH}_3)_2$;

Y is a single bond, O, S or NR^4 ,

each of p and q is zero or an integer from 1 to 5,

each of r and s is zero or an integer from 1 to 5,

each of R^1 and R^2 is independently selected from the group consisting of hydrogen, $(\text{C}_1\text{--}\text{C}_4)$ alkyl which may be hydroxy- or $(\text{C}_1\text{--}\text{C}_4)$ alkoxy- or $(\text{C}_1\text{--}\text{C}_4)$ alkylthio-substituted, hydroxy, $(\text{C}_1\text{--}\text{C}_4)$ alkoxy, $(\text{C}_1\text{--}\text{C}_4)$ alkylthio, amino and halogen,

each R^3 and R^4 is independently selected from the group consisting of hydrogen, $(\text{C}_1\text{--}\text{C}_4)$ alkyl, hydroxy-or alkoxy-or alkylthio-substituted $(\text{C}_1\text{--}\text{C}_4)$ alkyl, hydroxy, $(\text{C}_1\text{--}\text{C}_6)$ -alkoxy, $(\text{C}_1\text{--}\text{C}_6)$ -alkylthio and amino,

Q and I is independently selected from $-\text{CO}_2\text{H}$, $-\text{CONR}^{\text{I}}\text{R}^{\text{II}}$, $-\text{SO}_3\text{H}$ or $-\text{SO}_2\text{NR}^{\text{I}}\text{R}^{\text{II}}$ or an active derivative of $-\text{CO}_2\text{H}$ or $-\text{SO}_3\text{H}$ and $-\text{NR}^{\text{I}}\text{R}^{\text{III}}$

wherein R^{I} , R^{II} and R^{III} are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers and as well as nucleic acid binding moieties and

each of x_1 and y_1 is an integer of from 0 to 10.

add
a